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A new species of *Isospora* (Apicomplexa: Eimeriidae) from eastern coachwhip, *Coluber flagellum flagellum* (Reptilia: Ophidia) from Oklahoma

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Abstract

Between June 2013 and August 2014, four eastern coachwhips, *Coluber flagellum flagellum* were collected from Arkansas ($n = 2$) and Oklahoma ($n = 2$) and their faeces examined for coccidian parasites. One (25%) harboured an isosporan that we describe here as new. Oocysts of *Isospora kiamichiensis* sp. n. were spheroidal to subspheroidal with a uni-layered wall and measured (length $\times$ width, L $\times$ W) $25.0 \times 22.2 \mu\text{m}$, with an L/W ratio of 1.1. A micropyle, oocyst residuum and polar granule were absent. Sporocysts were ovoidal, $14.2 \times 9.8 \mu\text{m}$, with an L/W ratio of 1.5. A pronounced, button-like Stieda body was present as well as a substieda body. A sporocyst residuum was present as dispersed granules. This is the first isosporan and fourth coccidian reported from eastern coachwhip snakes. In addition, a single oocyst of an unknown choleoeimerian was recovered from this host.

Keywords

Apicomplexa; Coccidia; taxonomy; oocysts; Reptilia; Ophidia; *Isospora kiamichiensis*; Oklahoma; *Coluber flagellum flagellum*

Introduction

The eastern coachwhip, *Coluber* (= *Masticophis*) *flagellum flagellum* Shaw, 1802 is a large colubrid that ranges from North Carolina south to Florida and west to Missouri, Kansas, Arkansas, and eastern Oklahoma and Texas (Conant and Collins 1998). In Oklahoma, *M. f. flagellum* occurs east of Interstate 35 in open woodlands and prairies where it feeds on insects, rodents, snakes, birds and especially lizards (Sievert and Sievert 2011).

Three coccidians, *Eimeria zamenis* Phisalix, 1921, *Caryospora duszynskii* Upton, Current and Barnard, 1984 and *Caryospora masticophis* Upton, McAllister and Trauth, 1994 have

been reported from *C. f. flagellum* and western coachwhip, *C. f. testaceus*, all from specimens collected from Arkansas and Texas (see Duszynski and Upton 2009). However, we are not aware of any isosporans reported from *C. f. flagellum*. Herein, we provide a description of an isosporan from *C. f. flagellum* from Oklahoma.

Materials and Methods

Between June 2013 and August 2014, four adult *C. f. flagellum* were collected as fresh road-kills from Union County, Arkansas ($n = 2$) and Latimer ($n = 1$) and Pushmataha ($n = 1$) counties, Oklahoma, and examined for coccidia. Faecal samples from the rectum were placed in individual vials containing 2.5% (w/v) aqueous potassium dichromate ($K_2Cr_2O_7$). Samples were examined for coccidia by light microscopy after flotation in Sheather's sugar solution (specific gravity = 1.30). Measurements were taken on 22 sporulated oocysts from a single snake using a calibrated ocular micrometer and reported in micrometres (μm) with means followed by the ranges in parentheses; photographs were taken using Nomarski interference-contrast optics. Oocysts were ~7 days old when measured and photographed. Descriptions of oocysts and sporocysts follow guidelines of Wilber et al. (1998) as follows: oocyst length (L) and width (W), their ranges and ratios (L/W), micropyle (M), oocyst residuum (OR), polar granule(s) (PG), sporocyst length (L) and width (W), their ranges and ratio (L/W), sporocyst (SP), Stieda body (SB), substieda body (SSB), parastieda body (PSB), sporocyst residuum (SR), sporozoites (SZ) anterior (ARB) and posterior (PRB) refractile bodies, and nucleus (N). A host voucher was accessioned into the Arkansas State University Museum of Zoology, Herpetology Collection (ASUMZ), State University, Arkansas, USA. Photosyntypes of sporulated oocysts were accessioned into the Harold W. Manter Laboratory of Parasitology (HWML), Lincoln, Nebraska USA.

Results

One of four (25%) *C. f. flagellum* were found to be passing coccidian oocysts that we describe as new. In addition, the same snake was concurrently infected with a *Choleoeimeria* sp.

Isospora kiamichiensis sp. nov. (Figs. 1–4)

Description of sporulated oocyst: Oocyst with 2 sporocysts; shape spheroidal to subspheroidal; smooth uni-layered wall, colourless, ~0.5 thick, smooth; $L \times W$: 25.0×22.2 ($20\text{--}28 \times 19\text{--}24$); L/W : 1.1 (1.0–1.2); M, OR, PG, all absent.

Description of sporocyst and sporozoites: SP ovoidal with point on end opposite SB; smooth uni-layered wall, ~0.5 thick; $L \times W$: 13.9×9.4 ($11\text{--}16 \times 8\text{--}10$); L/W : 1.5 (1.2–1.7); SB pronounced, button-like, SSB body present, PSB body absent; SR: composed of large-sized granules in a compact mass or dispersed along perimeter of SP; SZ: (not measured) sausage-shaped with spheroidal ARB and PRB; single N slightly posterior to midpoint.

Taxonomic summary

Type host: Eastern coachwhip, *Coluber flagellum flagellum* Shaw, 1802 (Reptilia : Ophidia : Colubridae). Collected 7 August 2014.

Type specimens: Symbiotype host deposited in the ASUMZ as No. 33267. Photosyntype of sporulated oocyst deposited in the HWML as No. 75058.

Type locality: 3.2 km NE of Honobia off Indian Highway, Pushmataha County, Oklahoma, USA (34.550791°N, 94.970198°W, elev. 512 m).

Prevalence: In one of four (25%) of the type host; one of two (50%) Oklahoma.

Sporulation: Exogenous. All oocysts were passed unsporulated or partially sporulated and became fully sporulated within five days at ~23 C.

Prepatent and patent periods: Unknown.

Site of infection: Unknown, oocysts recovered from faeces.

Etymology: The specific epithet is given for the Kiamichi Mountains of southeastern Oklahoma where the type host was collected. Because of the restricted nature of land ownership in the region, these mountains remain rustic, rural and economically undeveloped, with few population centers.

Remarks: When the new species is compared to other isosporans described from related colubrid snakes, there are little or no morphological similarities. *Isoospora colubris* Matuschka, 1986, from the green whip snake, *Hierophis* (=Coluber) *viridiflavus* from Italy, possesses spheroidal oocysts that are considerably smaller (17.1 µm) (Matuschka 1986). Another isosporan, *Isoospora guzarica* Davronov, 1985, from the spotted desert racer, *Platycephalus* (=Coluber) *karelini* from Republic of Uzbekistan (Davronov 1985) is thought to be a *Sarcocystis* sp. (Upton et al. 1992) and considered a *species inquirenda* (see Duszynski and Upton 2009). In addition, *Isoospora wilsoni* Upton, McAllister, Trauth and Bibb, 1992 was described from the flathead snake, *Tantilla gracilis* from adjacent Arkansas. Its subspheroidal oocysts measures 17.2 × 17.0 (15–19 × 15–19) µm, considerably smaller than the new species. The only other valid isosporan from snakes include *Isoospora natricis* Yakimoff and Gousseff, 1935 from the European grass snake, *Natrix natrix* from Uzbekistan (Yakimoff and Gousseff 1935) which possesses much smaller spheroidal oocysts (14.4 µm). Two other isosporans, *Isoospora neivai* Pinto and Maciel 1929 from the jaracaca, *Bothrops jaracaca* from Brazil (Pinto and Maciel 1929) and *Isoospora guersae* Yakimoff and Matschoulsky, 1937 from the Levantine viper, *Macrovipera lebetina* from Uzbekistan (Yakimoff and Matschoulsky, 1937) are considered pseudoparasites, thus *species inquirenda* (Duszynski and Upton 2009).

In addition to the above mentioned isosporans, there have been two other species of *Isoospora* previously described from snakes: *Isoospora cenchoae* Asmundsson, Upton and Freed, 2001 from Amazon basin tree snake (*Imantodes lentiferus*) and *Isoospora dipsasi* Asmundsson, Upton and Freed, 2001 from vermiculate snail-eater (*Dipsas vermiculata*), both from Ecuador (Asmundsson et al. 2001). Neither of these have any morphological similarities with the new species.

We also report that the same host was concurrently infected with a choleoeimerian (Fig. 5) having a single elongate oocyst that measured $28.4 \times 15.1 \mu\text{m}$ ($L/W = 1.9$) and sporocysts that were $9.1 \times 7.7 \mu\text{m}$ ($L/W = 1.2$). Our measurement for oocyst length is smaller than those for *E. zamenis* previously reported from *M. f. flagellum* from an unknown locality (Roudabush 1937) but fall within those for kingsnakes, *Lampropeltis* spp. from Illinois (Anderson et al. 1968) and racers, *Coluber* spp. from Iowa (Wacha and Christiansen 1974); however, *E. zamenis* is likely composed of multiple species (Duszynski and Upton 2009), and further study with additional oocysts and possibly DNA sequencing will be necessary to help unravel this enigma.

Discussion

We here describe a new species of *Isospora* from an eastern coachwhip snake, but with some caution because (1) it was found in only one snake, (2) we do not have tissues for study of endogenous stages, and (3) there is the possibility of pseudoparasitism. Unfortunately, tissues were not available from this road-killed snake because they were in a state of decomposition when examined. In addition, because coachwhips are known to feed on a variety of reptiles, particularly lizards (Trauth and McAllister, 1995), this isosporan could be a pseudoparasite. And, only was snake was found to be passing oocysts and it would have been ideal to have additional infected snakes. Given this latter limitation, however, it is particularly noteworthy that in our previous studies on coccidia of coachwhip snakes from Arkansas, Oklahoma and Texas (Upton and McAllister, 1990; Upton et al., 1994; McAllister et al. 1995) only two of 40 individuals were found to be infected with three different species of coccidia, so prevalence may be quite low in these snakes.

Although no previous isosporans have been reported from Oklahoma snakes, there is potential for additional coccidians to be described from the state. In fact, McAllister et al. (2014) recently described a new species of *Isospora* from the ground skink, *Scincella lateralis* from adjacent Arkansas. In addition, McAllister et al. (2012) described a new species of *Caryospora* from *T. gracilis* from Oklahoma.

The state of Oklahoma supports 99 species of reptiles in a wide variety of diverse habitats ranging from coastal plains to high mesas (Sievert and Sievert 2011). It is noteworthy that about 90% of the reptiles in the state have not yet been surveyed for coccidia and what already has been reported about the other 10% is few and only includes snakes as hosts (Table 1). Indeed, we have just scratched the surface of the known coccidians of the state's herpetofauna and it is very likely that species new to science will be discovered in the course of future surveys, expanding our knowledge of the coccidia of Oklahoma's reptiles.

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Figs. 1-3.

Nomarski interference-contrast photomicrographs of oocysts of *Isospora kiamichiensis* n. sp. **1.** Subspheroidal oocyst and Stieda body (SB). **2.** Spheroidal oocyst with point on sporocyst (PT) and substieda body (SSB). **3.** Another subspheroidal oocyst showing unlayered oocyst wall (OW) and end view of sporocysts (SP). Scale bars = 10 μ m for all figures.

Fig. 4.
Composite line drawing of oocyst of *Isospora kiamichiensis* n. sp. Scale bar = 10 μ m.

Fig. 5. Nomarski interference-contrast photomicrograph of oocyst of *Choleoeimeria* sp. Scale bar = 10 μ m.

Table 1

Coccidia reported from Oklahoma snakes.

Coccidian and host	County locale	Prevalence*	Reference
<i>Caryospora duszynskii</i>			
<i>Lampropeltis calligaster</i>	not given	1/1 (100%)	McAllister et al. (2011)
<i>Pantherophis emoryi</i>	Greer	2/2 (100%)	McAllister et al. (1995, 2011)
<i>Eimeria choctawensis</i>			
<i>Tantilla gracilis</i>	Choctaw	1/15 (7%)	McAllister et al. (2012)
<i>Eimeria striatula</i>			
<i>Virginia striatula</i>	McCurtain	2/2 (100%)	McAllister et al. (1995)
<i>Isospora kiamichiensis</i>			
<i>Coluber flagellum</i>	Pushmataha	1/4 (25%)	This study
<i>Sarcocystis</i> sp.			
<i>L. calligaster</i>	McCurtain	2/4 (50%)	McAllister et al. (2013)
<i>Pantherophis obsoletus</i>	Le Flore	2/13 (15%)	McAllister et al. (2013)

* Number infected/number examined (%).